a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (*n*) of the column as follows:

$$n = 5.545\left(\frac{t_R}{w_h}\right)^2$$

where:
n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:
$\overline{X}$ is the mean of *N* individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using two injections of the same volume (100 microliters) of the working standard solution followed by one injection of the same volume (100 microliters) of the sample solution.

[50 FR 48398, Nov. 25, 1985; 51 FR 1367, Jan. 13, 1986]

§ 436.361 High-performance liquid chromatographic assay for aztreonam.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (*T*), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:
$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (*n*) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute; and

w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj}=Retention time of a solute eluting after i (t_{Rj} is larger than t_{Ri});

t_{Ri}=Retention time of any solute;

w_i=Width of peak at baseline of any solute; and

w_j=Width of peak at baseline of any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[52 FR 4611, Feb. 13, 1987; 52 FR 8550, Mar. 18, 1987]

§ 436.362 Thin-layer chromatographic test for free erythromycin content in erythromycin estolate bulk.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top.

(2) *Plates.* Use a 20- by 20-centimeter precoated silica gel 60 F–254 thin-layer chromatography plate. Before using, place the plate in an unlined developing chamber containing approximately 100 milliliters of anhydrous methanol and allow the solvent front to travel to the top of the plate, marking the direction of travel. Remove the plate and allow to drip dry. Store in a dry place.

(b) *Reagents*—(1) *Developing solvent.* Mix 15 milliliters of chloroform and 85 milliliters of anhydrous methanol. Use fresh developing solvent for each test.

(2) *Spray solution.* Dissolve 150 milligrams of xanthydrol in a mixture of 7.5 milliliters of glacial acetic acid and 92.5 milliliters of 37 percent hydrochloric acid.

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Prepare a solution of the sample in anhydrous methanol to contain 10 milligrams per milliliter.

NOTE: It is advisable to prepare the sample and standard solutions immediately before spotting to minimize the possibility of degradation in solution.)

(2) *Standard solution.* Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 1 milligram per milliliter. Weigh 99.5, 99.0, and 97.0 milligrams of erythromycin estolate (propionyl erythromycin lauryl sulfate) reference standard and transfer to separate 10-milliliter volumetric flasks. To these flasks add 0.5, 1.0, and 3.0 milliliters, respectively, of the 1-milligram-per-milliliter solution of erythromycin base reference standard and dilute to volume with anhydrous methanol. These solutions contain, respectively, 0.5 percent, 1.0 percent, and 3.0 percent erythromycin base in erythromycin estolate. Prepare a solution of erythromycin estolate reference standard in anhydrous methanol to contain 10 milligrams per milliliter. Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 0.1 milligram per milliliter.

(d) *Procedure.* Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a